Lanes c and d of the electrophoretic gel display the transcription products typical of runoff reactions counting all 4 rNTPs: there are abortive transcripts ranging up to 8 bases in length and a 59-base runoff product. The 4mer length the sequences of the poly-G transcripts and the abortive transcripts made in the presence of all 4 rNTPs (labeled "4H", "5H", etc.) diverge and no longer co-migrate with the poly-G transcripts of equivalent size.

When rATP is omitted from the transcription reactions (lanes e and f) normal elongation of the initially synthesized GGG trimer cannot occur. There are no heterogenous sequence abortive transcripts or 59 base runoff products made and instead long poly-G transcript s, as in lane a, are made. Adding dATP to reactions lacking rATP does not change the transcripts produced by the w.t. enzyme (lane g). However, with the mutant enzyme we observed that addition of dATP (lane h) allows synthesis a long runoff transcript as well as synthesis of heterogenous sequence abortive transcripts that do not co-migrate with the poly-G transcripts. Observation of an abortive transcript in lane h running near the position of the "4H" band in lane d confirms extension of the GGG trimer with an A but note that the major 4mer transcript in lane h migrates close to, but not precisely with, the major 4mer in lane d or in adjacent lane i. This is consistent with the expectation that these 4mers will have identical sequence and length but different structure (i.e., rGrGrGrA in lanes d or i; rGrGrGdA in lane h). It should also be noted that <u>some</u> poly-G transcript synthesis <u>is</u> observed in lane h. For example, in lane h we observe both a heterogeneous sequence 4mer migrating near the "4H" position and a smaller amount of 4mer band migrating at the "4G" position. When 4 rNTPs are present

(lanes c or d) the synthesis of poly-G transcripts is more completely suppressed. This indicates that dATP is utilized by Y639F, but not as efficiently as rATP.

When rCTP is omitted from the reaction, transcripts terminate predominately at the 6mer length because rCMP is normally first incorporated at position 7 (lanes i, j). Addition of dCTP does not allow extension of the 6mer in reactions with the w.t. enzyme (lane k). However, addition of dCTP to reactions with Y639F allows extension beyond the 6mer length and synthesis of the runoff transcript (lane l). Again, the following should be noted: 1. The transcripts larger than 6 bases do not co-migrate with their counterparts in lanes c or d consistent with the expected structural difference despite length and sequence identity, 2. there is more termination at the 6- and 7mer points in lane l than in lanes c or d, indicating that Y639F uses dCTP well, but not as efficiently as it utilizes rCTP.

In lanes m and n UTP was omitted from the reactions. Lanes q-t show a 10-fold longer exposure of lanes m-p. Within the set of 4 NTPs, UTP is unique on this template since it first becomes incorporated into the transcript at the 13 base position. This corresponds to a transcript length <u>subsequent</u> to the transition form abortive to processive transcription. As a consequence of this transition, the ternary complex becomes more stable (Martin, <u>et al</u>., 1988). Therefore, when transcript extension is blocked during the processive phase of transcription, the stalled ternary complex does not rapidly dissociate (Shi, <u>et al</u>., 1988). Instead it remains stalled on the template, near the promoter, and blocks reinitiation. For this reason we observe a large decrease in the overall amount of

transcription when UTP is omitted from the reaction in lanes m and n.

A longer exposure of lanes m and n (lanes q, r) does, however, reveal transcription products of the expected structure. When TTP is added to these reactions (lanes o, p, s, t) synthesis of the 59 base runoff transcript is observed with both the w.t. and Y639F mutant.

The ability of the w.t. enzyme to extend transcripts with TTP when it is unable to extend transcripts with the other dNTPs is also likely to be related to unique position at which UTP/TTP first becomes incorporated into the transcript. The amount of transcript termination or extension that occurs with a particular dNTP depends simply on the relative rates of ternary complex dissociation or dNMP incorporation (McClure and Chow, 1980). During abortive transcription complex, dissociation must be more rapid than the rate at which the w.t. enzyme incorporates dNMPs into its transcripts. During processive transcription even the expected slow rate of dNMP incorporation by the w.t. enzyme must be competitive with the slow rate of dissociation of the stable elongation complex, and an ability of the w.t. enzyme to incorporate dNMPs becomes manifest. Because elongation during the processive phase of transcription is fast (~230 bases/sec, Golomb and Chamberlin, 1974), while initiation and progression through abortive transcription is slow (Martin and Coleman, 1987; Martin, et al., 1988), elongation of the transcript from 11 to 59 bases is expected to contribute less than 10% to the time required for transcript synthesis. As a consequence the phase of transcription during which TTP is incorporated may not be the rate limiting step in synthesis of the runoff transcript even for the w.t. enzyme. These considerations